09-12-00

Customer Number: 000959

DIVISIONAL-CONTINUATION APPLICATION TRANSMITTAL FORM UNDER RULE 1.53(b) (former Rule 1.60)

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION:		PRIOR APPLICATION SERIAL NUMBER: 09/469,636	PRIOR APPLICATION FILING DATE: DECEMBER 22, 1999
AHN-001DV1	CLASS:	SUBCLASS:	EXAMINER:	ART UNIT:

ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
WASHINGTON, DC 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: September 11, 2000 Mailing Label Number: EL 011 359 939 US

I hereby certify that this 37 CFR 1.53(b) request and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Viriato 6 Cardoso — Name of Person Mailing Paper

Signature of Person Mailing Paper

Dear Sir:

This is a request for filing a ☐ continuation ☒ divisional application under 37 CFR 1.53(b), of pending prior application serial no. 09/469,636 filed on December 22, 1999, of Winfried Edelmann, Richard D. Kolodner, Jeffrey W. Pollard, and Raju S. Kucherlapati entitled *MSH5 ABLATED MICE AND USES THEREFOR.*

1. ☒ Enclosed is a copy of the latest inventor signed application, including the oath or declaration as originally filed. The copy of the enclosed papers is as follows:
   - ☒ 22 page(s) of specification
   - ☒ 2 page(s) of claims
   - ☒ 1 page(s) of abstract
   - ☒ 8 sheet(s) of drawing (Figures 1-6)
   - ☒ 10 page(s) of an executed declaration and power of attorney.

   I hereby verify that the attached papers are a true copy of the prior complete application serial no. 09/469,636 as originally filed on December 22, 1999.

2. ☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27, a copy of which is enclosed, was filed in the prior application and such status is still proper and desired (37 CFR 1.28(a)).

3. ☒ The filing fee is calculated below:

	NUMBER OF CLAIMS FILED			NUMBER EXTRA
TOTAL	* 10	MINUS	** 20	=
INDEP.	* 1	MINUS	*** 3	=
☐ MULTIPLE DEPENDENT CLAIMS				

SMALL ENTITY		OR	OTHER THAN A SMALL ENTITY	
RATE	FEE		RATE	FEE
x 9 =	$.00		x 18 =	$.00
x 39 =	$.00		x 78 =	$.00
+130 =	$.00		+ 260 =	$.00
BASIC FEE	$.00		BASIC FEE	$690.00
TOTAL	$0.00	OR	TOTAL	$690.00

4. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 12-0080. A duplicate copy of this sheet is enclosed.

5. ☒ A check in the amount of $690.00 is enclosed for payment of the filing fee.

6. ☒ Cancel in this application original claims 1-12 and 15-21 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

7. ☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claims in the prior application.)

8. ☒ Amend the specification by inserting before the first line the sentences: "This application is a divisional application of serial no. 09/469,636 filed on December 22, 1999, pending. The contents of all of the aforementioned application(s) are incorporated herein by reference."

9. ☐ Please abandon said prior application as of the filing date accorded this application. A duplicate copy of this transmittal is enclosed for filing in the prior application file. (May be used if signed by person authorized by §1.138 and before payment of base issue fee.)

10. ☐ Transfer the drawings from the pending prior application to this application.

11. ☐ Priority of application serial no. _____ filed on _____ in _____ is claimed under 35 U.S.C. §119.
    - ☐ The certified copy has been filed in prior application serial no. _____ filed on _____.
    - ☐ The certified copy will follow.

12. ☒ The prior application is assigned of record to Albert Einstein College of Medicine of Yeshiva University and Dana-Farber Cancer Institute, Inc.

13. ☐ A _____ month extension of time has been submitted in the parent application Serial No. in order to establish copendency with the present application.

14. ☒ Also enclosed is an Associate Power of Attorney (including Appendix A).

15. ☒ The power of attorney in the prior application is to Amy E. Mandragouras.
    a. ☒ The power appears in the original papers in the prior application.
    b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
    c. ☐ A new power has been executed and is attached.

16. ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Amy E. Mandragouras at **Customer Number: 000959** whose address is:

Lahive & Cockfield, LLP
28 State Street
Boston, Massachusetts 02109

17. ☒ Any requests for extensions of time necessary in a parent application for establishing copendency between this application and a parent application are hereby requested and the Commissioner is authorized to charge any fee associated with such an extension to Deposit Account No. 12-0080.

18. ☒ Pursuant to 37 CFR 1.821(e), the computer readable form of the sequence listing for this new application is to be identical with the computer readable form of application serial no. 09/469,636. Please use the computer readable form of application serial no. 09/469,636 in lieu of filing a duplicate computer readable form in this application. Pursuant to 37 CFR 1.821(f), the content of the paper copy of the sequence listing for this new application and the computer readable form of application serial no. 09/469,636 are the same.

September 11, 2000
Date

Maria C. Laccotripe, Ph.D.
Agent for Applicants
Limited Recognition Under 37 C.F.R. 10.9(b)

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, Massachusetts 02109
Tel. (617) 227-7400

☐ inventor(s) ☐ filed under §1.34(a)
☐ assignee of complete interest
☒ attorney or agent of record

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Winfried Edelmann, Richard D. Kolodner, Jeffrey W. Pollard, and Raju S. Kucherlapati

Divisional of Serial No.: 09/469,636

Filed: Herewith

For: *METHODS FOR IDENTIFYING COMPOUNDS WHICH MODULATE THE ACTIVITY OF MSH5* (*As amended*)

Attorney Docket No.: AHN-001DV1

Art Unit:

Examiner:

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

CERTIFICATE OF EXPRESS MAILING

"Express Mail" mailing label number EL 011 359 939 US

Date of Deposit September 11, 2000

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Viriato 6 Cardoso Viriato 6 Cardoso

Signature Please Print Name of Person Signing

09469636 091100

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination of the above-identified application, please amend the application as follows:

In the specification:

At page 1, line 1, please replace "MSH5 ABLATED MICE AND USES THEREFOR" with -- METHODS FOR IDENTIFYING COMPOUNDS WHICH MODULATE THE ACTIVITY OF MSH5--;

At page 25, line 1, please replace "MSH5 ABLATED MICE AND USES THEREFOR" with -- METHODS FOR IDENTIFYING COMPOUNDS WHICH MODULATE THE ACTIVITY OF MSH5--;

At page 25, lines 4-6, please replace the abstract with --The present invention provides methods for identifying compounds which modulate the activity of MSH5.--.

In the claims:

Please cancel claims 1-12 and 15-21 without prejudice and amend claim 14 as follows:

14. **(Amended)** The method of claim 13, wherein said compound inhibits the activity of MSH5 [is inhibited].

Please add new claims 22-29 as follows:

22. The method of claim 14, wherein said compound is a contraceptive agent.

23. The method of claim 13, wherein said compound is capable of modulating MSH5 expression.

24. The method of claim 23, wherein said compound is an antisense MSH5 nucleic acid molecule.

25. The method of claim 13, wherein said compound is a small molecule.

26. The method of claim 13, wherein said compound is an MSH5 antibody.

27. The method of claim 13, wherein said compound is a peptide.

28. The method of claim 13, wherein said compound is a peptidomimetic.

29. The method of claim 13, wherein said compound has an effect on an MSH5 substrate.

REMARKS

Claims 1-21 were pending in the present application. Claims 1-12 and 15-21 have been canceled without prejudice for further prosecution in one or more divisional applications. Claim 14 has been amended and new claims 22-29 have been added. Accordingly, claims 13, 14, and 22-29 are currently pending. Support for the new claims can be found throughout the specification, including the originally filed claims. Specifically, support for claim 22 can be found at, for example page 25, line 6 of the specification. Support for claims 23, 25, 27, 28 and 29 can be found at, for example page 14, lines 30-35 of the specification. Support for claim 24 can be found at, for example page 15, line 27 of the specification. Support for claim 26 can be found at, for example page 15, line 21 of the specification.

No new matter has been added. Any amendments to and/or cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

CONCLUSION

In view of the amendments and remarks set forth above, it is respectfully submitted that this application is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with Applicants' Agent would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at (617) 227-7400.

Respectfully submitted,
LAHIVE & COCKFIELD, LLP

Maria C. Laccotripe, Ph.D.
Agent for Applicant
Limited Recognition Under 37 C.F.R.§ 10.9(b)

28 State Street
Boston, Massachusetts 02109
telephone: (617) 227-7400
facsimile: (617) 742-4214
Date: September 11, 2000

APPENDIX A

13. A method for identifying a compound which modulates the activity of MSH5, comprising:

a) contacting MSH5 with a test compound; and

b) determining the effect of the test compound on the activity of MSH5 to, thereby, identify a compound which modulates MSH5 activity.

14. The method of claim 13, wherein said compound inhibits the activity of MSH5.

22. The method of claim 14, wherein said compound is a contraceptive agent.

23. The method of claim 13, wherein said compound is capable of modulating MSH5 expression.

24. The method of claim 23, wherein said compound is an antisense MSH5 nucleic acid molecule.

25. The method of claim 13, wherein said compound is a small molecule.

26. The method of claim 13, wherein said compound is an MSH5 antibody.

27. The method of claim 13, wherein said compound is a peptide.

28. The method of claim 13, wherein said compound is a peptidomimetic.

29. The method of claim 13, wherein said compound has an effect on an MSH5 substrate.

MSH5 ABLATED MICE AND USES THEREFOR

Related Applications

This application claims priority to U.S. provisional Application No. 60/113,487, filed on December 22, 1998, incorporated herein in its entirety by this reference.

Government Funding

Work described herein was supported by funding from the National Institute of Health. The United States Government has certain rights in the invention.

Field of the Invention

The invention relates to animals in which the MutS homolog 5 (MSH5) gene is misexpressed and methods of using such animals or cells derived therefrom, e.g., in methods of evaluating fertility treatments.

Background of the Invention

MutS homolog 5 (*MSH5*) is a member of a family of proteins that are known to be involved in DNA mismatch repair (Modrich, P. & Lahue (1996) *Annu. Rev. Biochem.* 65, 101-133; Kolodner, R. (1996) *Genes Dev.* 10, 1433-1442). Germ line mutations in *MSH2*, *MLH1* and *MSH6* cause hereditary non-polyposis colon cancer (HNPCC) or Lynch syndrome (Leach, F.S. *et al.* (1993) *Cell* 75, 1215-1225; Bronner, C.E. *et al.* (1994) *Nature* 368, 258-261; Papadopoulos, N. *et al.* (1994) *Science* 263, 1625-1629; Akiyama, Y. *et al.* (1997) *Cancer Res.* 57, 3920-3923; Miyaki, M. *et al.* (1997) *Nature Genet.* 17, 271-272). Inactivation of *Msh2*, *Mlh1*, *Msh6* and *Pms2* in mice leads to hereditary predisposition to intestinal and other cancers (de Wind, N.et al. (1995) *Cell* 82, 321-330; Reitmair, A.H. *et al.* (1995) *Nature Genet.* 11, 64-70). Early studies in yeast revealed a role for some of these proteins, including MSH5, in meiosis (Hollingsworth, N.M.,et al. (1995) *Genes & Development* 9, 1728-1739 ; Ross-Macdonald, P. & Roeder, G.S. (1994) *Cell* 79, 1069-1080). Gene targeting studies in mice confirmed roles for MLH1 and PMS2 in mammalian meiosis (Baker, S.M. *et al.* (1995) *Cell* 82, 309-320; Edelmann, W. *et al.* (1996) *Cell* 85, 1125-1134; Baker, S.M. *et al. Nature Genet.* 13, 336-342).

Summary of the Invention

The present invention is based, at least in part, on the generation of animals which are homozygous for a null mutation in the MutS homolog 5 (MSH5) gene and the

observation that these animals are sterile. Accordingly, the invention features, a non-human animal, in which the gene encoding the MutS homolog 5 (MSH5) protein is misexpressed.

In preferred embodiments the animal, which is preferably a transgenic animal, is a mammal, e.g., a non human primate or a swine, e.g., a miniature swine, a monkey, a goat, or a rodent, e.g., a rat, but preferably a mouse.

In preferred embodiments, expression of the gene encoding the MSH5 protein is decreased as compared to the wild-type animal. For example, the levels of the MSH5 protein can be suppressed by, at least, 50%, 60%, 70%, 80%, 90%, or 100% as compared to the wild-type animal.

In preferred embodiments, misexpression of the gene encoding the MSH5 protein is caused by disruption of the MSH5 gene. For example, the MSH5 gene can be disrupted through removal of DNA encoding all or part of the protein.

In preferred embodiments, the animal can be heterozygous or homozygous for a misexpressed MSH5 gene, e.g., it can be a transgenic animal heterozygous or homozygous for an MSH5 transgene.

In preferred embodiments, the animal is a transgenic mouse with a transgenic disruption of the MSH5 gene, preferably an insertion or deletion, which inactivates the gene product.

In another aspect, the invention features, a nucleic acid molecule which, when introduced into an animal or cell, results in the misexpression of the MSH5 gene in the animal or cell. In preferred embodiments, the nucleic acid molecule, includes an MSH5 nucleotide sequence which includes a disruption, e.g., an insertion or deletion and preferably the insertion of a marker sequence. For example, a nucleic acid molecule can be the targeting construct shown in Figure 1.

In another aspect, the invention features, a method of evaluating a fertility treatment. The method includes: administering the treatment to an MSH5 misexpressing animal, e.g., a transgenic mouse, or a cell therefrom; and determining the effect of the treatment on a fertility indication, e.g., sperm count, testicular size, or oocyte morphology, to thereby evaluate the treatment for fertility. The method may be performed in vivo or in vitro.

In preferred embodiments, the animal or cell is an animal or cell described herein. In other preferred embodiments, the method uses a transgenic mouse in which the expression of the MSH5 gene is inhibited. In yet other preferred embodiments, the method uses a cell derived from a transgenic mouse in which the expression of the MSH5 gene is inhibited.

In another aspect, the invention features, a method for identifying a compound which modulates the activity of MSH5. The method includes contacting MSH5 with a test compound and determining the effect of the test compound on the activity of MSH5 to, thereby, identify a compound which modulates MSH5 activity. In preferred embodiments, the activity of MSH5 is inhibited.

In another aspect, the invention features, a method for modulating the activity of MSH5. The method includes contacting MSH5 or a cell expressing MSH5 with a compound which binds to MSH5 in an amount sufficient (e.g., a sufficient concentration) to modulate the activity of MSH5. In preferred embodiments, the activity of MSH5 is inhibited, e.g., the method can be used in contraception.

In another aspect, the invention features, a method of identifying a subject having or at risk of developing a fertility disease or disorder. The method includes obtaining a sample from said subject; contacting the sample with a nucleic acid probe or primer which selectively hybridizes to MSH5 and determining whether aberrant MSH5 expression or activity exists in the sample, thereby, identifying a subject having or at risk of developing a fertility disease or disorder.

In another aspect, the invention features, an isolated cell, or a purified preparation of cells, from an MSH5 misexpressing animal, e.g., an MSH5 misexpressing animal described herein. In preferred embodiments, the cell is a transgenic cell, in which the gene encoding the MSH5 protein is misexpressed. The cell, preferably a transgenic cell can be an oocyte or a spermatocyte.

In preferred embodiments, the cell is heterozygous or homozygous for the transgenic mutant gene.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 is a schematic of the generation of *Msh5* null mice. *Figure 1A* depicts the gene targeting strategy. *Figure 1B* is a depiction of a Southern blot of tail DNA digested with *NsiI*. DNA analysis of 606 offspring from heterozygote matings produced 184 $Msh5^{+/+}$, 275 $Msh5^{+/-}$ and 147 $Msh5^{-/-}$, confirming the Mendelian transmission of the mutant allele. *Figure 1C* is a depiction of a Northern blot of RNA from $Msh5^{+/+}$ and $Msh5^{-/-}$ mouse testes with different probes. *Figure 1D* is a depiction of a Western blot of proteins from male testes with anti-MSH5 antibody.

Figure 2 depicts the disruption of spermatogenesis in *Msh5* $^{-/-}$ males. *Figure 2A* is a depiction of the mRNA expression of *Msh5* (upper panel) and actin (lower panel) in testes from wild-type males between the ages of 8 days and 29 days, and in adult wild-type and *Msh5* $^{-/-}$ males. *Figure 2B-E* is a depiction of H&E stained sections of adult testis from wild-type (*B, D*) and *Msh5* $^{-/-}$ (*C, E*) males showing loss of spermatocytes beyond zygonema in *Msh5*-deficient males. Le, Leydig cell; S, Sertoli cell; A, type A spermatogonia; B, type B spermatogonia; PL, pre-Leptotene; L, Leptotene spermatocyte; Z, Zygotene spermatocyte; P, Pachytene spermatocyte; RS, round spermatid; ES, elongated spermatid; Sp, spermatozoa. *Figure 2F,G* is a depiction of the immunolocalization of germ cells using anti-GCNA1 antibody (red immunoreactive protein against a light blue counterstain) on sections from wild-type (*F*) and *Msh5* $^{-/-}$ (*G*) testes from 29 day old males showing abundant spermatocytes, spermatids and spermatozoa in wild-type testes and a few GCNA1-positive cells in the MSH5-deficient testes. (For *B* and *C*, scale bar = 100 μm; for *D-G*, scale bar = 25 μm).

Figure 3 depicts the progressive depletion of germ cells in *Msh5* $^{-/-}$ males during development. *Figure 3A, B, E, F, I, J* is a depiction of germ cell immunolocalization using the anti-GCNA1 antibody of testes from wild-type (*A, E, I*) and *Msh5* $^{-/-}$ (*B, F, J*) males showing the rapid depletion of germ cells from day 17pp onwards in *Msh5*-deficient mice in contrast to the increasing density and variety of spermatogenic cells in the seminiferous tubules of *Msh5*+/+ males. *Figure 3C, D, G, H, K, L* is a depiction of TUNEL staining of testes from wild-type (*C, G, K*) and *Msh5* $^{-/-}$ males (*D, H, L*) showing continuous apoptosis from day 17 pp onwards compared to the very low level of apoptosis in tubules from wild-type males over the same time frame. (Scale bar = 100 μm.)

Figure 4 depicts the disruption of meiosis prior to synapsis in *Msh5* spermatocytes. *Figure 4 A-C* is a depiction of silver-stained spermatocytes from wild-type (*A*) and *Msh5* $^{-/-}$ (*B,C*) testes showing complete failure of pairing (*B*) or some partial pairing (*C*) in the absence of MSH5. Arrowheads in panel (*C*) indicate chromosomes exhibiting partial pairing. Note that many of these chromosomes appear to be unequally paired.

Figure 5 depicts the loss of oocytes and subsequent ovarian degeneration in *Msh5* $^{-/-}$ females. *Figure 5A,B* depicts ovaries from day 3 pp wild-type (*A*) and *Msh5* $^{-/-}$ (*B*) females showing oocytes stained with GCNA1. *Figure 5c* depicts the entire ovary from a day 25 pp *Msh5* $^{-/-}$ female (H&E staining) containing only 3 follicles and degenerating tissue. *Figure 5D,E* is a depiction of H&E stained ovaries from adult wild-type (*D*) and *Msh5* $^{-/-}$ females (*E*) showing complete loss of oocytes and ovarian

architecture in the absence of *Msh5*. B, ovarian bursa; Ov, oviduct. In all cases, scale bar = 200 μm. *Figure 5F* is a depiction of the expression of ZP3 and Actin in ovaries of wild-type and *Msh5* $^{-/-}$ ovaries on day 25 pp and in the adult.

Figure 6 shows that the disruption of oogenesis in *Msh5* $^{-/-}$ females leads to a failure of folliculogenesis. *Figure 6*A-D depicts ovaries from e18 wild-type (*A,B*) and *Msh5* $^{-/-}$ (*C,D*) embryos showing oogonia stained with anti-GCNA1 (*A,C*) or H&E localization of meiotic chromosome detail (*C,D*). *Figure 6*E-H depicts GCNA1 localization of oocytes in ovaries from day 3 pp wild-type (*E,F*) and *Msh5* $^{-/-}$ (*G,H*) females. Arrowheads indicate pachytene oocytes (punctate red staining of nucleus compared to solid red staining of pre-pachytene oocytes), arrows indicate the appearance of the earliest primordial follicles. *Figure 6I, and 6J,* is a depiction of GCNA1 localization of oocytes in ovaries from day 6pp wild-type (*I,J*) females (overstained to stain oocytes in meiotic arrest). Arrows indicate primordial follicles; o, oocyte. For *A, C, E, G, and I* scale bar = 100 μm; for *B, D, F, H, and J* scale bar = 25 μm.

Detailed Description

The present invention is based, at least in part, on the generation of animals which are homozygous for a null mutation in the MutS homolog 5 (MSH5) gene and the observation that these animals are sterile. Accordingly, the invention features, a non-human animal, in which the gene encoding the MutS homolog 5 (MSH5) protein is misexpressed. In preferred embodiments the animal, is preferably a transgenic animal.

As used herein, a "transgenic animal" includes an animal, e.g., a non-human mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a mouse, in which one or more, and preferably essentially all, of the cells of the animal include a transgene. The transgene is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, e.g., by microinjection, transfection or infection, e.g., by infection with a recombinant virus. The term genetic manipulation includes the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

As used herein, the term "rodent" refers to all members of the phylogenetic order *Rodentia*.

As used herein, the term "misexpression" includes a non-wild type pattern of gene expression. Expression as used herein includes transcriptional, post transcriptional, e.g., mRNA stability, translational, and post translational stages. Misexpression includes: expression at non-wild type levels, i.e., over or under expression; a pattern of

expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus. Misexpression includes any expression from a transgenic nucleic acid. Misexpression includes the lack or non-expression of a gene or transgene, e.g., that can be induced by a deletion of all or part of the gene or its control sequences.

As used herein, the term "knockout" refers to an animal or cell therefrom, in which the insertion of a transgene disrupts an endogenous gene in the animal or cell therefrom. This disruption can essentially eliminate MSH5 in the animal or cell.

In preferred embodiments, misexpression of the gene encoding the MSH5 protein is caused by disruption of the MSH5 gene. For example, the MSH5 gene can be disrupted through removal of DNA encoding all or part of the protein.

In preferred embodiments, the animal can be heterozygous or homozygous for a misexpressed MSH5 gene, e.g., it can be a transgenic animal heterozygous or homozygous for an MSH5 transgene.

In preferred embodiments, the animal is a transgenic mouse with a transgenic disruption of the MSH5 gene, preferably an insertion or deletion, which inactivates the gene product.

In another aspect, the invention features, a nucleic acid molecule which, when introduced into an animal or cell, results in the misexpression of the MSH5 gene in the animal or cell. In preferred embodiments, the nucleic acid molecule, includes an MSH5 nucleotide sequence which includes a disruption, e.g., an insertion or deletion and preferably the insertion of a marker sequence. The nucleotide sequence of the wild type MSH5 is known in the art and described in, for example, Winand, N.J.et al. (1998) *Genomics* 53, 69-80, the contents of which are incorporated herein by reference. For example, the nucleic acid molecule can be the targeting construct, shown in Figure 1.

As used herein, the term "marker sequence" refers to a nucleic acid molecule that (a) is used as part of a nucleic acid construct (e.g., the targeting construct) to disrupt the expression of the gene of interest (e.g., the MSH5 gene) and (b) is used to identify those

cells that have incorporated the targeting construct into their genome. For example, the marker sequence can be a sequence encoding a protein which confers a detectable trait on the cell, such as an antibiotic resistance gene, e.g., neomycin resistance gene, or an assayable enzyme not typically found in the cell, e.g., alkaline phosphatase, horseradish peroxidase, luciferase, beta-galactosidase and the like.

As used herein, "disruption of a gene" refers to a change in the gene sequence, e.g., a change in the coding region. Disruption includes: insertions, deletions, point mutations, and rearrangements, e.g., inversions. The disruption can occur in a region of the native MSH5 DNA sequence (e.g., one or more exons) and/or the promoter region of the gene so as to decrease or prevent expression of the gene in a cell as compared to the wild-type or naturally occurring sequence of the gene. The "disruption" can be induced by classical random mutation or by site directed methods. Disruptions can be transgenically introduced. The deletion of an entire gene is a disruption. Preferred disruptions reduce MSH5 levels to about 50% of wild type, in heterozygotes or essentially eliminate MSH5 in homozygotes.

In another aspect, the invention features, a method of evaluating a fertility treatment. The method includes: administering the treatment to an MSH5 misexpressing animal or a cell therefrom; and determining the effect of the treatment on a fertility indication, to thereby evaluate the treatment for fertility. The method may be performed in vivo or in vitro. As used herein, the term "fertility indication" includes any parameter related to fertility, e.g., sperm count, testicular size, or oocyte morphology.

As used herein, "administering a treatment to an animal or cell" is intended to refer to dispensing, delivering or applying a treatment to an animal or cell. In terms of the therapeutic agent, the term "administering" is intended to refer to contacting or dispensing, delivering or applying the therapeutic agent to an animal by any suitable route for delivery of the therapeutic agent to the desired location in the animal, including delivery by either the parenteral or oral route, intramuscular injection, subcutaneous/intradermal injection, intravenous injection, buccal administration, transdermal delivery and administration by the intranasal or respiratory tract route.

In preferred embodiments, the animal or cell is an animal or cell described herein. In other preferred embodiments, the method uses a transgenic mouse in which the expression of the MSH5 gene is inhibited. In yet other preferred embodiments, the method uses a cell derived from a transgenic mouse in which the expression of the MSH5 gene is inhibited.

In another aspect, the invention features, a method for identifying a compound which modulates the activity of MSH5. The method includes contacting MSH5 with a

test compound and determining the effect of the test compound on the activity of MSH5 to, thereby, identify a compound which modulates MSH5 activity. In preferred embodiments, the activity of MSH5 is inhibited.

As used herein, the term "compound" includes any agent, e.g., peptides, peptidomimetics, small molecules, or other drugs, which bind to MSH5 proteins, have a stimulatory or inhibitory effect on, for example, MSH5 expression or MSH5 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a MSH5 substrate.

In another aspect, the invention features, a method for modulating the activity of MSH5. The method includes contacting MSH5 or a cell expressing MSH5 with a compound which binds to MSH5 in an amount sufficient to modulate the activity of MSH5. In preferred embodiments, the activity of MSH5 is inhibited, e.g., in contraception.

As used herein, the term "contraception" includes the prevention of fertilization, preferably without destroying fertility.

In another aspect, the invention features, a method of identifying a subject having or at risk of developing a fertility disease or disorder. The method includes obtaining a sample from said subject; contacting the sample with a nucleic acid probe or primer which selectively hybridizes to MSH5 and determining whether aberrant MSH5 expression or activity exists in the sample, thereby, identifying a subject having or at risk of developing a fertility disease or disorder.

As used herein, the term "fertility disease or disorder" includes any disease disorder or condition which affects fertilization. Fertility diseases include conditions in which the development of the gametes, i.e., the ovum and the sperm, is abnormal, as well as conditions in which a fetus cannot be carried to term. Examples of such fertility disorders include low sperm count, habitual abortion, and abnormal ovulation.

In another aspect, the invention features, an isolated cell, or a purified preparation of cells, from an MSH5 misexpressing animal, e.g., an MSH5 misexpressing animal described herein. In preferred embodiments, the cell is a transgenic cell, in which the gene encoding the MSH5 protein is misexpressed. The cell, preferably a transgenic cell is an oocyte or a spermatocyte.

In preferred embodiments, the cell is heterozygous or homozygous for the transgenic mutant gene.

As used herein, the term "transgenic cell" refers to a cell containing a transgene.

As used herein, "purified preparation" is a preparation which includes at least 10%, more preferably 50%, yet more preferably 90% by number or weight of the subject cells.

The present invention is described in further detail in the following subsections.

Preparation of MSH5 Targeting Constructs

The MSH5 nucleotide sequence to be used in producing the targeting construct is digested with a particular restriction enzyme selected to digest at a location(s) such that a new DNA sequence encoding a marker gene can be inserted in the proper position within this MSH5 nucleotide sequence. The marker gene should be inserted such that it can serve to prevent expression of the native gene. The position will depend on various factors such as the restriction sites in the sequence to be cut, and whether an exon sequence or a promoter sequence, or both is (are) to be interrupted (i.e., the precise location of insertion necessary to inhibit MSH5 gene expression). In some cases, it will be desirable to actually remove a portion or even all of one or more exons of the gene to be suppressed so as to keep the length of the targeting construct comparable to the original genomic sequence when the marker gene is inserted in the targeting construct. In these cases, the genomic DNA is cut with appropriate restriction endonucleases such that a fragment of the proper size can be removed.

The marker sequence can be any nucleotide sequence that is detectable and/or assayable. For example, the marker gene can be an antibiotic resistance gene or other gene whose expression in the genome can easily be detected. The marker gene can be linked to its own promoter or to another strong promoter from any source that will be active in the cell into which it is inserted; or it can be transcribed using the promoter of the MSH5 gene. The marker gene can also have a polyA sequence attached to the 3' end of the gene; this sequence serves to terminate transcription of the gene. For example, the marker sequence can be a protein that (a) confers resistance to antibiotics or other toxins; e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, and neomycin, hygromycin, or methotrexate for mammalian cells; (b) complements auxotrophic deficiencies of the cell; or (c) supplies critical nutrients not available from complex media.

After the MSH5 DNA sequence has been digested with the appropriate restriction enzymes, the marker gene sequence is ligated into the MSH5 DNA sequence using methods known to the skilled artisan and described in Sambrook et al., *Molecular*

Cloning A Laboratory Manual, 2nd Ed., ed., Cold Spring Harbor Laboratory Press: 1989, the contents of which are incorporated herein by reference.

Preferably, the ends of the DNA fragments to be ligated are compatible; this is accomplished by either restricting all fragments with enzymes that generate compatible ends, or by blunting the ends prior to ligation. Blunting is performed using methods known in the art, such as for example by the use of Klenow fragment (DNA polymerase I) to fill in sticky ends.

The ligated targeting construct can be inserted directly into embryonic stem cells, or it may first be placed into a suitable vector for amplification prior to insertion. Preferred vectors are those that are rapidly amplified in bacterial cells such as the pBluescript II SK vector (Stratagene, San Diego, CA) or pGEM7 (Promega Corp., Madison, WI).

Construction of Transgenic Mice

Transfection of Embryonic Stem Cells

Mouse embryonic stem cells (ES cells) can be used to generate the transgenic (e.g., knockout) MSH5 mice. Any ES cell line that is capable of integrating into and becoming part of the germ line of a developing embryo, so as to create germ line transmission of the targeting construct is suitable for use herein. For example, a mouse strain that can be used for production of ES cells, is the 129J strain. A preferred ES cell line is murine cell line D3 (American Type Culture Collection catalog no. CRL 1934). The cells can be cultured and prepared for DNA insertion using methods known in the art and described in Robertson, *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. IRL Press, Washington, D.C., 1987, in Bradley et al., *Current Topics in Devel. Biol.*, 20:357-371, 1986 and in Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986, the contents of which are incorporated herein by reference.

The knockout construct can be introduced into the ES cells by methods known in the art, e.g., those described in Sambrook et al. Suitable methods include, electroporation, microinjection, and calcium phosphate treatment methods.

The targeting construct to be introduced into the ES cell is preferably linear. Linearization can be accomplished by digesting the DNA with a suitable restriction

endonuclease selected to cut only within the vector sequence and not within the MSH5 gene sequence.

After the introduction of the targeting construct, the cells are screened for the presence of the construct. The cells can be screened using a variety of methods. Where the marker gene is an antibiotic resistance gene, the cells can be cultured in the presence of an otherwise lethal concentration of antibiotic. Those cells that survive have presumably integrated the knockout construct. A southern blot of the ES cell genomic DNA can also be used. If the marker gene is a gene that encodes an enzyme whose activity can be detected (e.g., beta-galactosidase), the enzyme substrate can be added to the cells under suitable conditions, and the enzymatic activity can be analyzed.

To identify those cells with proper integration of the targeting construct, the DNA can be extracted from the ES cells using standard methods. The DNA can then be probed on a southern blot with a probe or probes designed to hybridize in a specific pattern to genomic DNA digested with particular restriction enzymes. Alternatively, or additionally, the genomic DNA can be amplified by PCR with probes specifically designed to amplify DNA fragments of a particular size and sequence such that, only those cells containing the targeting construct in the proper position will generate DNA fragments of the proper size.

Injection/Implantation of Embryos

Procedures for embryo manipulation and microinjection are described in, for example, *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., 1986, the contents of which are incorporated herein by reference). Similar methods are used for production of other transgenic animals. In an exemplary embodiment, mouse zygotes are collected from six week old females that have been super ovulated with pregnant mares serum (PMS) followed 48 hours later with human chorionic gonadotropin. Primed females are placed with males and checked for vaginal plugs on the following morning. Pseudo pregnant females are selected for estrus, placed with proved sterile vasectomized males and used as recipients. Zygotes are collected and cumulus cells removed. Furthermore, blastocytes can be harvested. Pronuclear embryos are recovered from female mice mated to males. Females are treated with pregnant mare serum, PMS, to induce follicular growth and human chorionic gonadotropin, hCG, to induce ovulation. Embryos are recovered in a Dulbecco's modified phosphate buffered saline (DPBS) and maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum.

Microinjection of an MSH5 targeting construct can be performed using standard micro manipulators attached to a microscope. For instance, embryos are typically held in 100 microliter drops of DPBS under oil while being microinjected. DNA solution is microinjected into the male pronucleus. Successful injection is monitored by swelling of the pronucleus. Recombinant ES cells can be injected into blastocytes, using similar techniques. Immediately after injection embryos are transferred to recipient females, e.g. mature mice mated to vasectomized male mice. In a general protocol, recipient females are anesthetized, paralumbar incisions are made to expose the oviducts, and the embryos are transformed into the ampullary region of the oviducts. The body wall is sutured and the skin closed with wound clips.

Screening for the Presence of the Targeting Construct

Transgenic (e.g., knockout) animals can be identified after birth by standard protocols. DNA from tail tissue can be screened for the presence of the targeting construct using southern blots and/or PCR. Offspring that appear to be mosaics are then crossed to each other if they are believed to carry the targeting construct in their germ line to generate homozygous knockout animals. If it is unclear whether the offspring will have germ line transmission, they can be crossed with a parental or other strain and the offspring screened for heterozygosity. The heterozygotes are identified by southern blots and/or PCR amplification of the DNA.

The heterozygotes can then be crossed with each other to generate homozygous transgenic offspring. Homozygotes may be identified by southern blotting of equivalent amounts of genomic DNA from mice that are the product of this cross, as well as mice that are known heterozygotes and wild type mice. Probes to screen the southern blots can be designed as set forth above.

Other means of identifying and characterizing the knockout offspring are known in the art. For example, northern blots can be used to probe the mRNA for the presence or absence of transcripts encoding either the gene knocked out, the marker gene, or both. In addition, western blots can be used to assess the level of expression of the gene knocked out in various tissues of these offspring by probing the western blot with an antibody against the protein encoded by the gene knocked out (e.g., the MSH5 protein), or an antibody against the marker gene product, where this gene is expressed. Finally, *in situ* analysis (such as fixing the cells and labeling with antibody) and/or FACS (fluorescence activated cell sorting) analysis of various cells from the offspring can be

performed using suitable antibodies to look for the presence or absence of the targeting construct gene product.

Other Transgenic Animals

The transgenic animal used in the methods of the invention can be a mammal; a bird; a reptile or an amphibian. Suitable mammals for uses described herein include: ruminants; ungulates; domesticated mammals; and dairy animals. Preferred animals include: goats, sheep, camels, cows, pigs, horses, oxen, llamas, chickens, geese, and turkeys. Methods for the preparation and use of such animals are known in the art. A protocol for the production of a transgenic pig can be found in White and Yannoutsos, *Current Topics in Complement Research: 64th Forum in Immunology*, pp. 88-94; US Patent No. 5,523,226; US Patent No. 5,573,933; PCT Application WO93/25071; and PCT Application WO95/04744. A protocol for the production of a transgenic rat can be found in Bader and Ganten, *Clinical and Experimental Pharmacology and Physiology*, Supp. 3:S81-S87, 1996. A protocol for the production of a transgenic cow can be found in *Transgenic Animal Technology, A Handbook*, 1994, ed., Carl A. Pinkert, Academic Press, Inc. A protocol for the production of a transgenic sheep can be found in *Transgenic Animal Technology, A Handbook*, 1994, ed., Carl A. Pinkert, Academic Press, Inc.

Uses of MSH5 Transgenic Mice

MSH5 misexpressing animals, e.g., mice, or cells can be used to screen treatments for MSH5-related disorders, e.g., fertility disorders. The candidate treatment can be administered over a range of doses to the animal or cell. Efficacy can be assayed at various time points for the effects of the treatment on the disorder being evaluated.

Such treatments can be evaluated by determining the effect of the treatment on a fertility indication. Such parameters include sperm count, testicular size, or oocyte morphology. For example, treatment of a fertility condition includes treatment of ovary degeneration in the animal to, thereby, identify treatments suitable for administration to human subjects.

Methods of the invention can be used to study cells derived from the MSH5 ablated animals in order to define the mechanism of MSH5 function in cell processes, e.g., meiosis. For example, cells can be isolated from MSH5 misexpressing animals and used to identify agents that act downstream from MSH5 in the MSH5 pathway or in independent pathways.

Candidate Treatments

The candidate treatment, which is evaluated using methods described herein, can include: (a) the administration of a therapeutic agent (e.g., a drug, a chemical, an antibody, a protein, a nucleic acid or other substance) to a MSH5 misexpressing animal or cell; (b) the administration of a diet regimen to an MSH5 misexpressing animal; (c) the administration of ionizing radiation to an MSH5 misexpressing animal or cell. Any combination of the afore-mentioned treatments can be administered to an MSH5 misexpressing animal or cell. The treatment can be administered prior to, simultaneously and/or after the onset of the disorder or condition, for which the candidate treatment is being evaluated. The therapeutic agent can be administered to a MSH5 misexpressing animal, orally, parenterally or topically.

Predictive/Diagnostic Assays

The present invention also pertains to the field of predictive medicine in which diagnostic and prognostic assays are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining MSH5 protein and/or nucleic acid expression as well as MSH5 activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant MSH5 expression or activity, e.g., infertility. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with MSH5 protein, nucleic acid expression or activity. For example, mutations in an MSH5 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby phophylactically treat an individual prior to the onset of a disorder characterized by or associated with MSH5 protein, nucleic acid expression or activity.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to MSH5 proteins, have a stimulatory or inhibitory effect on, for example, MSH5 expression or MSH5 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of an MSH5 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of an MSH5 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of an MSH5 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating MSH5 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with MSH5 or an agent that modulates one or more of the activities of the MSH5 protein. An agent that modulates MSH5 protein activity can be a nucleic acid or a protein, a naturally-occurring target molecule of an MSH5 protein an MSH5 antibody, an MSH5 agonist or antagonist, a peptidomimetic of an MSH5 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more MSH5 activities. Examples of such stimulatory agents include active MSH5 protein and a nucleic acid molecule encoding MSH5 that has been introduced into the cell. In another embodiment, the agent inhibits one or more MSH5 activites. Examples of such inhibitory agents include antisense MSH5 nucleic acid molecules, anti-MSH5 antibodies, and MSH5 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a MSH5 protein or nucleic acid molecule, e.g., a fertility disorder. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) MSH5 expression or activity. In another embodiment,

the method involves administering an MSH5 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant MSH5 expression or activity.

Stimulation of MSH5 activity is desirable in situations in which MSH5 is abnormally downregulated and/or in which increased MSH5 activity is likely to have a beneficial effect. For example, stimulation of MSH5 activity is desirable in situations in which a MSH5 is downregulated and/or in which increased MSH5 activity is likely to have a beneficial effect. Likewise, inhibition of MSH5 activity is desirable in situations in which MSH5 is abnormally upregulated and/or in which decreased MSH5 activity is likely to have a beneficial effect.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

Examples

Materials and Methods

Mouse *Msh5* cDNA cloning

The original segment of the mouse *Msh5* gene was obtained by PCR using BALB/c genomic DNA (Clontech) and primers GTGCTGTGGAATTCAGGATAC (sense; SEQ ID NO:1) and CCAGAACTCTCTGGAGAAGC (antisense; SEQ ID NO:2) based on the human cDNA sequence. The remainder of the mouse *Msh5* coding sequence was cloned by RT-PCR using the Advantage cDNA PCR Kit and gene-specific primers CTCCACTATCCACTTCATGCCAGATGC (sense; SEQ ID NO:3) and GCTGGGGAGGACACTGGAAGGACTCTCA (antisense, based on human 3'-untranslated cDNA sequence; SEQ ID NO:4).

The mouse *Msh5* genomic locus was cloned from a P1 mouse embryonic stem cell genomic library screened by Genome Systems, Inc. which yielded three clones 11051, 11052, and 11053.

Construction of the p*Msh5ex18* targeting vectors

A genomic *Msh5* fragment containing exon 18 was obtained by screening a mouse genomic Charon 35, 129/Ola phage library. A 3.8 kb HindIII fragment containing exon 18 was subcloned into pBluescript SK+/- and a 2.0 kb BglII PGKhygro

cassette was cloned into the AatII site at codon 528 in exon 18 using BglII/AatII adaptors. The resulting gene targeting clone was designated p*Msh5ex18*.

Electroporation of embryonic stem cells

The targeting vector p*Msh5ex18* (50ug) was electroporated into WW6 ES cells (described in Ioffe, E. *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92, 7357-7361) and hygromycin resistant colonies were isolated and screened by PCR using forward primer A 5'-AGCTGGAGAACCTGGACTCTC -3' (SEQ ID NO:5) and reverse primer B 5'-TGGAAGGATTGGAGCTACGG-3' (SEQ ID NO:6). Positive ES cell colonies were identified by a 1.5 kb PCR fragment specific for the targeting event. Six positive cell lines MSH5-1, MSH5-33, MSH5-41, MSH5-52, MSH5-58, and MSH5-109 were identified and the correct targeting event was shown by NsiI digestion of high molecular weight DNA and Southern Blot analysis using a 0.8kb EcoRI/HindIII probe directed at the 5' intron region between exons13 and 14 that is not included in the targeting vector.

Northern Blot Analysis

Four μg of polyA RNA from 24 day old males was separated on 1.0% Agarose Formaldehyde gels, transferred onto Nitrocellulose membrane and hybridized with an *Msh5* probe corresponding to exons 3 to 8, a probe spanning the complete mouse *Msh4* cDNA and a human β-actin probe.

Western blot analysis

For Western blot analysis equal amounts of protein from testes extracts of 23 day old males were separated on a 10% SDS-polyacrylamide gel and transferred onto a Immobilon-P (Millipore) membrane. The membrane was blocked in TBS, 0.1% Tween-20, 5% nonfat dry milk, 10% goat serum (Sigma) and incubated with 1:1,000 diluted primary anti-MSH5 antibody. Bound protein was detected by chemiluminescence using a 1:30,000 diluted goat anti-mouse IgG horseradish peroxidase conjugate (Sigma).

Histology

Ovaries from *Msh5* +/+ and *Msh5* $^{-/-}$ females between e18 and 5 wks postpartum (pp) were removed and fixed in Bouins or 4% buffered formalin for 30-360 minutes before transferring to 70% ethanol. Testes were fixed by transcardiac perfusion of 4% buffered formalin and then overnight in fresh fixative. All tissues were processed for histology by routine methods and were sectioned at 3 or 5 μm.

Chromosomes

Chromosome spreads were prepared according to the method of Counce and Meyer (described in Counce, S.J. & Meyer, G.F. (1973) *Chromosoma* 44, 231-253, the contents of which are incorporated herin by reference), with modifications. Spreads were then either silver stained in 50% silver nitrate at 65°C for 6 hours (for electron microscopy) or subjected to immunofluorescence localization of chromosomally-associated proteins, according to the method of Moens (described in Spyropoulos, B. & Moens, P.B. (1994) *Methods in Molecular Biology* 33, 131-139, the contents of which are incorporated herein by reference).

Example 1: Generation and Analysis of Msh5 $^{-/-}$ Mice

To assess the role of MSH5 in mammals, mice with a null mutation in *Msh5* were generated and characterized . *Msh5* $^{-/-}$ mice are viable but are sterile. Meiosis in these mice is severely affected owing to the disruption of chromosome pairing in prophase I. This meiotic failure leads to a diminution in testicular size and a complete loss of ovarian structures. These results show that normal MSH5 function is essential for meiotic progression and, in females, gonadal maintenance.

A mouse *Msh5* genomic clone was isolated and used to construct a gene targeting vector (see Figure 1*A*) that was used to generate mice from two ES cell lines with the modified *Msh5* locus (see Figure 1*B*). The mice transmitted the modified locus in a Mendelian fashion and homozygous Msh5$^{-/-}$ mice were viable. *Msh5* transcripts or protein were not detectable in testes of 24 day old mice (see Figure 1D). These data indicate that the modified *Msh5* locus does not encode a functional MSH5 protein. In the mouse testis, the first meiotic wave begins at day 11 pp (see Figure 2*A*), with prophase I commencing at day 13. *Msh5* is highly expressed in the gonads of humans and mice, and in the latter is co-incident with the onset of the meiotic wave. *Msh5*$^{-/-}$ males exhibited normal sexual behavior, but they were infertile due to the complete absence of epididymal spermatozoa. Examination of seminiferous tubules in *Msh5* $^{-/-}$ adult males revealed a severe disruption of spermatogenesis (see Figure 2*B, C*), causing a 70% reduction in testis size. Interstitial Leydig cells and tubular Sertoli cells are present in the mutant males, as are type A and B spermatogonia, but no normal pachytene spermatocytes are observed (see Figure 2*D-G*). At day 17 pp, the seminiferous epithelium of *Msh5* $^{-/-}$ males, are fairly densely packed, although early signs of germ cell loss are evident, both by reduced germ cell nuclear antigen 1 (GCNA1, described in Enders, G.C. & May, J.J. (1994) *Developmental Biology.* 163, 331-340, the contents of which are incorporated herein by reference) localization and by

increased apoptosis (see Figure 3*A, B, C, D*). By day 23 pp, the tubules of wild-type mice contain round spermatids (see Figure 3*E, G*). In contrast, elevated levels of apoptosis in *Msh5* $^{-/-}$ tubules leads to continued germ cell attrition (see Figure 3F, H) and by adulthood, almost the entire spermatogenic cell population is lost (see Figure 3I-L).

To analyze meiotic progression, meiotic chromosome spreads were examined at the light and electron microscope level. In 23 day old wild-type spreads, silver staining revealed a range of chromosomal configurations, including those at leptotene, zygotene, pachytene and diplotene (see Figure 4*A*). However, from four *Msh5* $^{-/-}$ males it was found that 588/602 (97.7%) spermatocytes contained no synapsed chromosomes (see Figure 4*B*) compared to >92% of wild-type cells (255/277) showing chromosomal configurations at zygotene and beyond. All of the spermatocytes from *Msh5* $^{-/-}$ males contained univalent chromosomes and condensation levels corresponding to zygotene/pachytene stages of meiosis. In the remaining 14 cells only 29 partially paired chromosomes were observed out of the expected 280 pairs (see Figure 4*C*). At least half of these (15/29) involved chromosomes of different lengths suggesting that this pairing is non-homologous.

The chromosomal association of SYCP1, SYCP3 and RAD51 proteins known to be required for recombination and formation of the synaptonemal complex (SC) (Moens, P.B. et al. (1998) *Current Topics in Molecular Biology* 37, 241-263; Plug, A.W.,et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 5920-5924) was also examined. Immunofluorescent localization of SYCP1 and SYCP3 on meiotic chromosomes using a combined antiserum demonstrated normal acquisition of SC in spermatocytes from wild-type males and identified pachytene spermatocytes as having 20 distinct condensed pairs of bivalents. In *Msh5* $^{-/-}$ spermatocytes, all of the chromosomes were clearly associated with the SYCP1/SYCP3 signal, indicating that axial element formation has been achieved but no condensed bivalents were observed. In *Msh5* $^{-/-}$ spermatocytes, RAD51 is localized in discrete foci along the univalent chromosomes, and the number and intensity of these foci appears greater in the majority of *Msh5* $^{-/-}$ cells than on leptotene or zygotene chromosomes from wild-type males and does not decline as observed in wild-type spermatocytes suggesting lack of progress towards pachytene. The presence of RAD51 on unsynapsed chromosomes mutant mice suggests that meiosis is initiated and that double strand breaks might proceed in the absence of MSH5.

To examine the role of MSH5 in female meiosis, ovarian function was assessed in *Msh5* $^{-/-}$ adults. The mutants did not mate with wild-type males, nor did they undergo normal estrous cycles. The *Msh5* $^{-/-}$ females have normally structured oviducts and uteri but lack discernible ovaries (see Figure 5*D,E*). Instead, the ovarian bursa of *Msh5*

females was empty or, more frequently, contained cystic structures with 1-4 cysts (see Figure 5*E*). At day 3 pp, the ovaries of *Msh5* $^{-/-}$ females contained fewer oocytes (see Figure 5*A,B*). By day 25 pp, the ovaries of *Msh5* $^{-/-}$ females were reduced to a small grouping of 1-3 follicles that appeared to be at post-antral stages of development, and occasionally contained oocytes (see Figure 5*C*) while wild-type ovaries have abundant primordial follicles. The presence of oocytes in day 25 pp *Msh5* $^{-/-}$ females was confirmed by RT-PCR detection of transcripts for the oocyte-specific protein, zona pellucida 3 (ZP3) described in Wassarman, P.M. (1998) *Annual Review of Biochemistry.* **57**, 415-442, the contents of which are incorporated herein by reference. However, in adults ZP3 transcripts could only be detected in wild-type ovaries (see Figure 5*F*). Thus, the ovaries of *Msh5* $^{-/-}$ females are normal size at birth, but degenerate progressively to become rudimentary, concomitant with the decline in oocyte numbers from before day 3 pp until adulthood.

Msh5 expression was examined in wild-type ovaries by RT-PCR. *Msh5* expression was detected in e16, e18 and day1 pp ovaries coincident with the initiation of meiosis in females and consistent with the possibility that MSH5 plays a direct role in ovarian meiosis. During late embryogenesis, the ovaries of homozygous mutant females contain normal numbers of oocytes (see Figure 6*A, C*). However, examination of H&E sections revealed subtle differences in chromosome structure between wildtype and *Msh5* $^{-/-}$ oocytes, characterized by clumping of nuclear contents in the homozygous mutant oocytes (see Figure 6*D*) compared to readily identifiable chromosomes in the wild-type oocytes (see Figure 6*B*). By day 3 pp, the number of oocytes in the ovaries of *Msh5* $^{-/-}$ females was dramatically lower than that in wild-type ovaries (see Figure 6*E,G*) and did not exhibit the GCNA1 staining characteristic of pachytene oocytes (see Figure 6*F,H*). By day 6 pp, large, primordial follicles containing readily identifiable oocytes were distributed throughout the ovary of wild-type females (see Figure 6*I, J*), while in ovaries from *Msh5* $^{-/-}$ females the oocyte pool was severely diminished.

The results show that MSH5 is required for chromosome pairing and/or synapsis. Mutations in the other mouse MutHLS genes, *Pms2* and *Mlh1*, which interact with MSH homologs, are also sterile due to meiotic abnormalities. However, the stage at which meiosis is aberrant in these mice is different. In *Pms2* $^{-/-}$ mice, chromosome pairing is disrupted but spermatids and spermatozoa, although abnormal, were observed. In *Mlh1* $^{-/-}$ mice, normal pairing was detected but post-pachytene meiotic stages were rarely observed. These results suggest that these proteins have distinct roles at different stages of meiosis.

In adult *Msh5* $^{-/-}$ females, the phenotype is even more dramatic than in males because of the complete loss of ovarian structures. Similar to the *Msh5* $^{-/-}$ males, the germ cells populate the genital ridge but the oocytes never progress beyond zygotene. The progressive loss of oocytes from e18 appears to result from meiotic failure and the activation of a checkpoint resulting in apoptosis, as seen in *Msh5* $^{-/-}$ spermatocytes. This results in an almost complete absence of oocytes by day 6 pp and the ovary begins to degenerate such that, in the adult, it is usually entirely absent or consists of a few large cysts. The degenerating oocytes fail to initiate folliculogenesis showing that there must be dialog between the oocyte and the surrounding stroma for this process and to maintain ovarian morphology. The phenotype of *Msh5* $^{-/-}$ females differs from that seen in *Dmc1* $^{-/-}$ mice which also show a failure of pairing/synapsis and oocyte loss in early neonatal life but retain at least a rudimentary ovary in adulthood. These differences suggest that either the requirement for MSH5 is slightly earlier than DMC1 or there is partial redundancy for DMC1 function.

There are similarities in the ovarian phenotype in female *Msh5*$^{-/-}$ mice and Turner syndrome patients. In both cases there is a rapid loss of oocytes during intrauterine and neonatal life and consequent ovarian degeneration. It is possible that the failure of homologous chromosome pairing, whether at the level of the X chromosome (as in Turner patients) or throughout the entire chromosome population (as in *Msh5*$^{-/-}$ oocytes) triggers an apoptotic checkpoint that ultimately results in complete ovarian degeneration.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS:

1. A non-human animal, in which the gene encoding the MSH5 gene is misexpressed.

2. The animal of claim 1, wherein said animal is a transgenic animal.

3. The animal of claim 2, wherein said transgenic animal is a mouse.

4. The animal of claim 1, wherein the MSH5 gene is disrupted by removal of DNA encoding all or part of the MSH5 protein.

5. The animal of claim 4, wherein said animal is homozygous for the disrupted gene.

6. The animal of claim 4, wherein said animal is heterozygous for the disrupted gene.

7. The animal of claim 1, wherein said animal is a transgenic mouse with a transgenic disruption of the MSH5 gene.

8. The animal of claim 7, wherein said disruption is an insertion or deletion.

9. A method of evaluating a fertility treatment, comprising:

administering said treatment to an MSH5 misexpressing animal or a cell therefrom and determining the effect of the treatment on a fertility indication, thereby evaluating said fertility treatment.

10. The method of claim 9, wherein said treatment is evaluated in vivo.

11. The method of claim 9, wherein said treatment is evaluated in vitro.

12. The method of claim 9, wherein said MSH5 misexpressing animal is a transgenic mouse.

13. A method for identifying a compound which modulates the activity of MSH5, comprising:

a) contacting MSH5 with a test compound; and

b) determining the effect of the test compound on the activity of MSH5 to, thereby, identify a compound which modulates MSH5 activity.

14. The method of claim 13, wherein the activity of MSH5 is inhibited.

15. A method for modulating the activity of MSH5 comprising contacting MSH5 or a cell expressing MSH5 with a compound which binds to MSH5 in a sufficient concentration to modulate the activity of MSH5.

16. The method of claim 15, wherein the activity of MSH5 is inhibited.

17. The method of claim 16, wherein said method is used in contraception.

18. A method of identifying a subject having or at risk of developing a fertility disease or disorder, comprising:

(a) obtaining a sample from said subject;

(b) contacting said sample with a nucleic acid probe or primer which selectively hybridizes to MSH5; and

(c) determining whether aberrant MSH5 expression or activity exists in said sample, thereby, identifying a subject having or at risk of developing a fertility disease or disorder.

19. An isolated cell, or a purified preparation of cells from an MSH5 misexpressing animal.

20. The cell of claim 19, wherein said cell is transgenic cell.

21. The cell of claim 20, wherein said transgenic cell is a mouse cell.

MSH5 ABLATED MICE AND USES THEREFOR

Abstract of the Invention

An animal, e.g., transgenic mouse, in which the MSH5 gene is misexpressed. The animal is useful for screening treatments for a number of conditions. Methods for identifying contraceptive agents are also described.

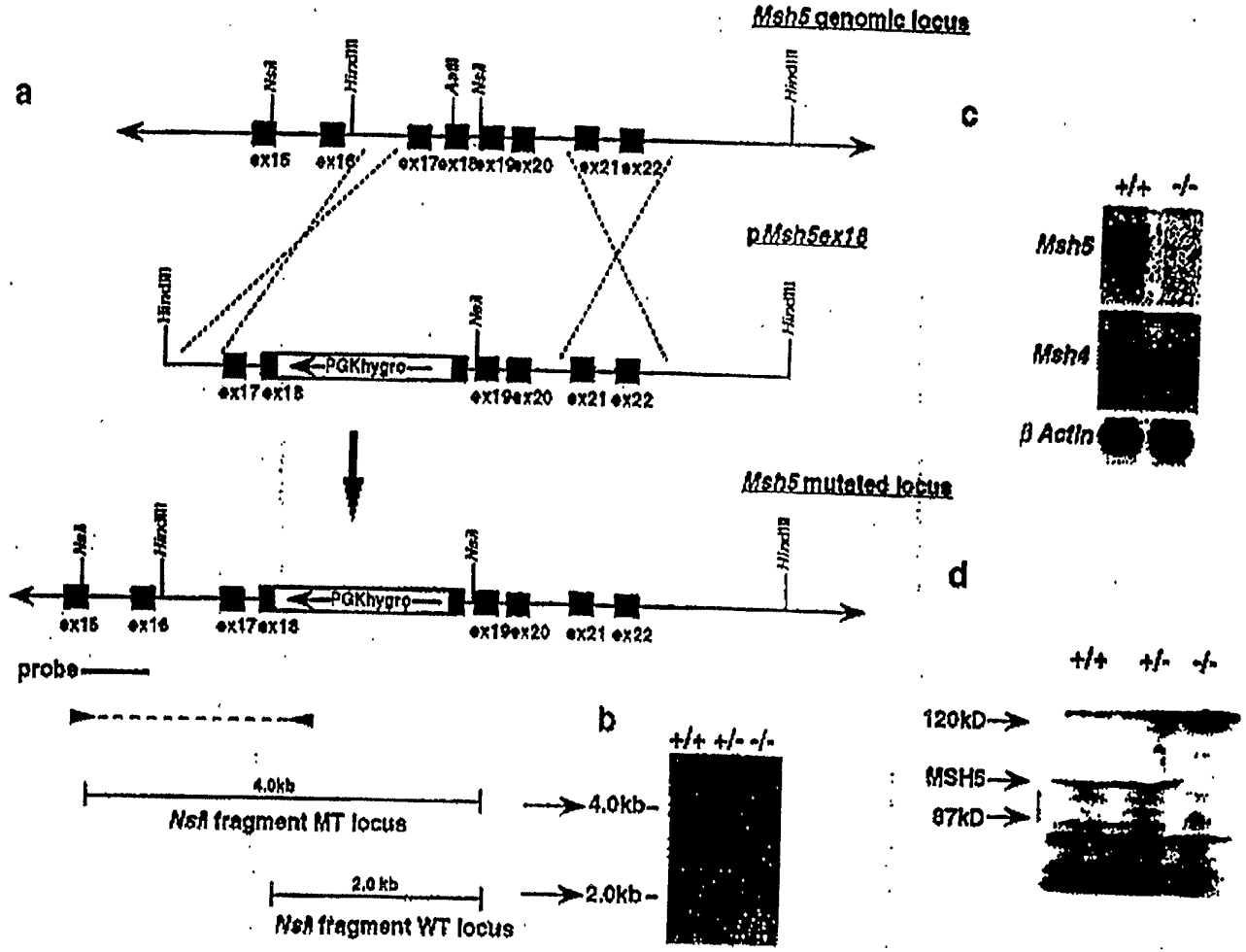

FIGURE 1

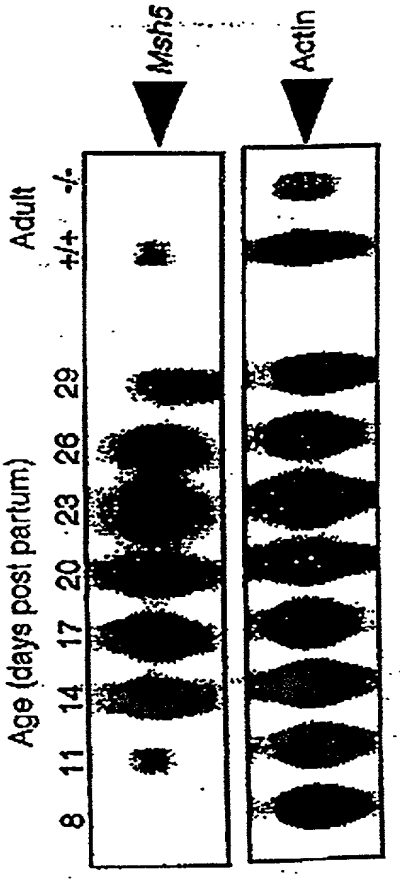

FIGURE 2A

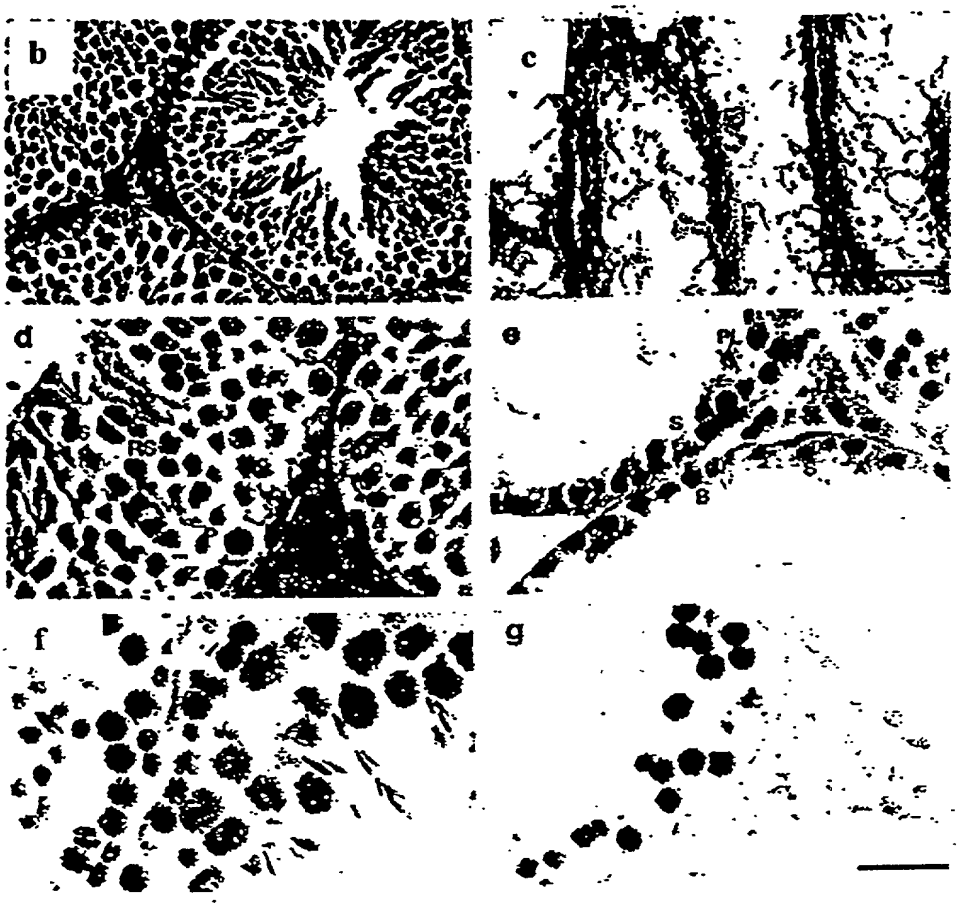

FIGURE 2B-G

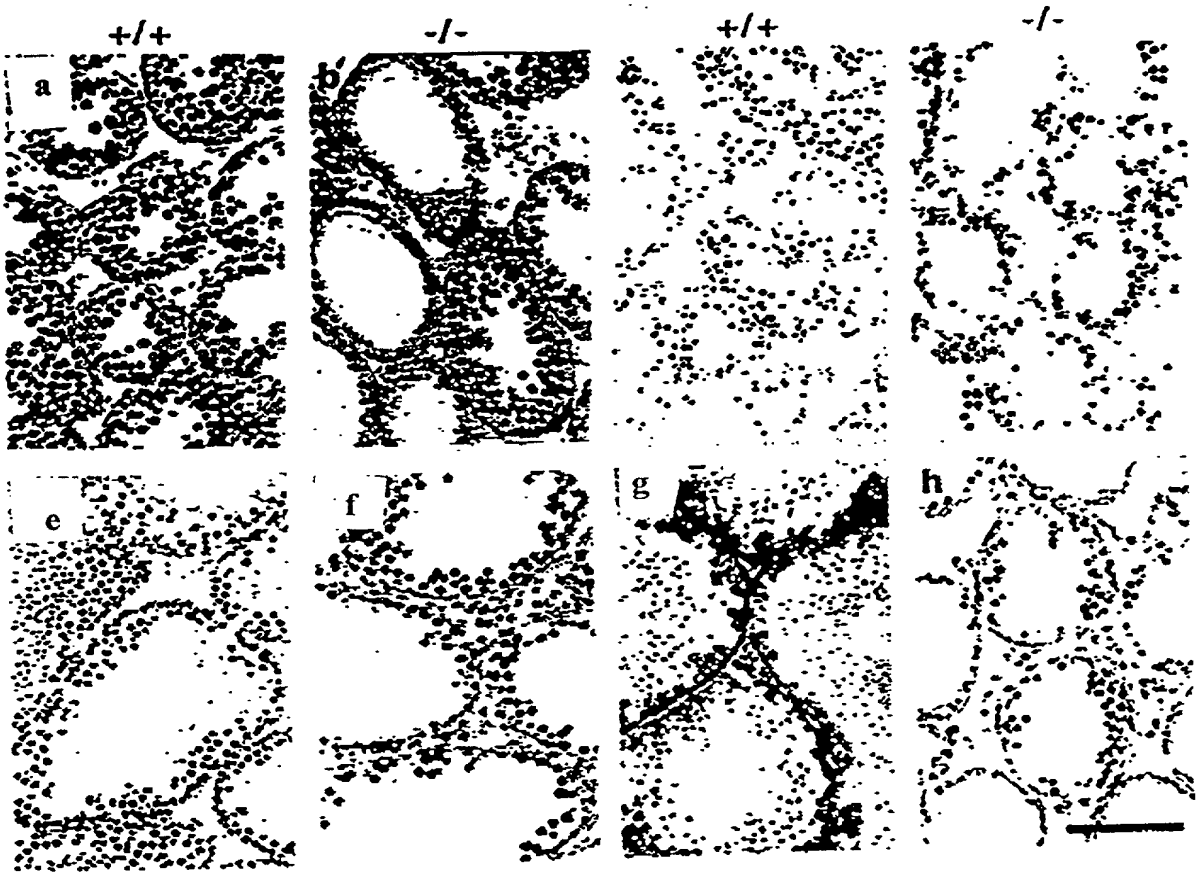

FIGURE 3

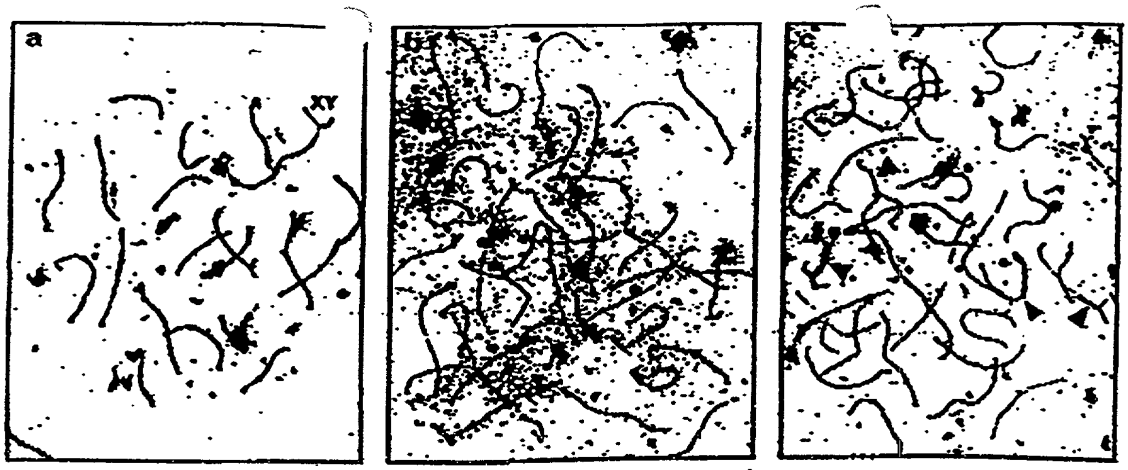

FIGURE 4

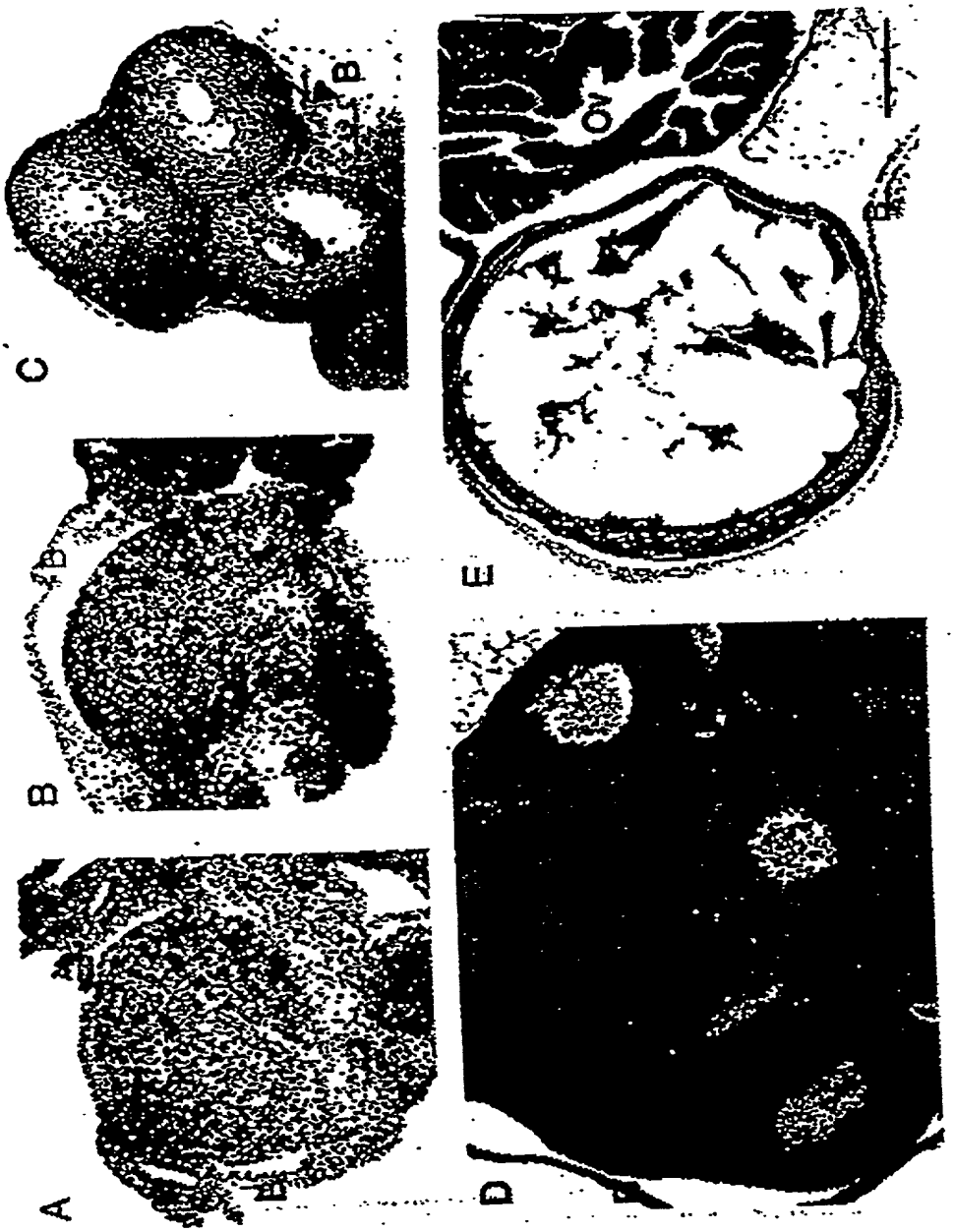

FIGURE 5A-E

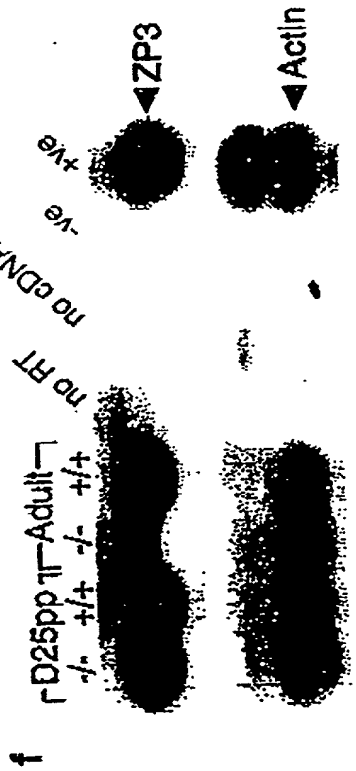

FIGURE 5F

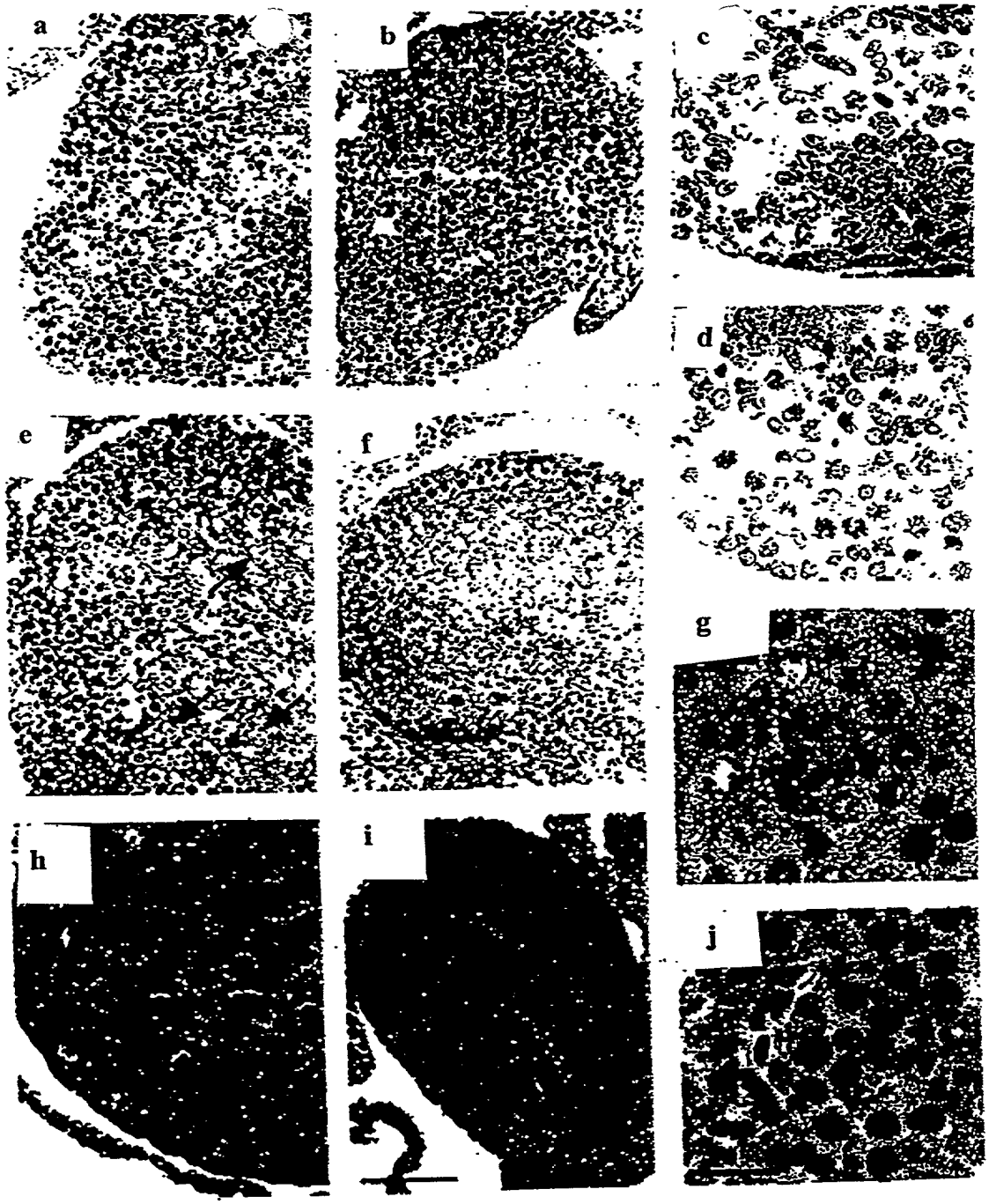

FIGURE 6

Customer Number: 000959

Attorney's
Docket
Number AHN-001

Declaration, Petition and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MSH5 ABLATED MICE AND USES THEREFOR
the specification of which

(check one)

___ is attached hereto.

X was filed on December 22, 1999 as

Application Serial No. 09/469,636

and was amended on _____.
(if applicable)

I do not know and do not believe that the subject matter of this application was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date of this application, or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date of this application on an application filed more than twelve months (six months if this application is for a design) before the filing of this application; and I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

CLAIM OF BENEFIT OF EARLIER FOREIGN APPLICATION(S)

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application(s) for patent or inventor's certificate filed by me on the same subject matter having a filing date before that of the application(s) from which priority is claimed.

Check one:

X no such applications have been filed.

_ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)
60/113,487	December 22, 1998

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)	(Filing Date)	(Status) (patented,pending,aband.)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

W. Hugo Liepmann	Reg. No. 20,407	Catherine J. Kara	Reg. No. 41,106
James E. Cockfield	Reg. No. 19,162	Jeanne M. DiGiorgio	Reg. No. 41,710
Thomas V. Smurzynski	Reg. No. 24,798	Megan E. Williams	Reg. No. 43,270
Ralph A. Loren	Reg. No. 29,325	Nicholas P. Triano III	Reg. No. 36,397
Giulio A. DeConti, Jr.	Reg. No. 31,503	Peter C. Lauro	Reg. No. 32,360
Ann Lamport Hammitte	Reg. No. 34,858	Timothy J. Douros	Reg. No. 41,716
Elizabeth A. Hanley	Reg. No. 33,505	John L. Welch	Reg. No. 28,129
Amy E. Mandragouras	Reg. No. 36,207	DeAnn F. Smith	Reg. No. 36,683
Anthony A. Laurentano	Reg. No. 38,220	William D. DeVaul	Reg. No. 42,483
Jane E. Remillard	Reg. No. 38,872	David J. Rikkers	Reg. No. 43,882
Jeremiah Lynch	Reg. No. 17,425	Chi Suk Kim	Reg. No. 42,728
Kevin J. Canning	Reg. No. 35,470		
David A. Lane, Jr.	Reg. No. 39,261		

Send Correspondence to Amy E. Mandragouras at **Customer Number: 000959** whose address is:

Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109

Direct Telephone Calls to: Amy E. Mandragouras , (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Winfried Edelmann
Inventor's signature Winfried Edelmann Date 4/11/00
Residence 5800 Arlington Ave., Apt. 10H Bronx, New York 10471
Citizenship German
Post Office Address (if different)

Full name of second inventor, if any Richard D. Kolodner	
Inventor's signature	Date
Residence 13468 Kibbings Rd. San Diego, California 92130	
Citizenship U.S.	
Post Office Address (if different)	

Full name of third inventor, if any Jeffrey W. Pollard	
Inventor's signature	Date 4/11/00
Residence 275 W. Ninety-sixth Street. New York, New York 10025	
Citizenship U.S.	
Post Office Address (if different)	

Full name of fourth inventor, if any Raju S. Kucherlapati	
Inventor's signature	Date 4/11/00
Residence Five Gracie Lane Darrien, Connecticut 06820	
Citizenship U.S.	
Post Office Address (if different)	

Customer Number: 000959

Attorney's
Docket
Number AHN-001

Declaration, Petition and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MSH5 ABLATED MICE AND USES THEREFOR
the specification of which

(check one)

___ is attached hereto.

X was filed on December 22, 1999 as

Application Serial No. 09/469,636

and was amended on _____.
(if applicable)

I do not know and do not believe that the subject matter of this application was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date of this application, or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date of this application on an application filed more than twelve months (six months if this application is for a design) before the filing of this application; and I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

CLAIM OF BENEFIT OF EARLIER FOREIGN APPLICATION(S)

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application(s) for patent or inventor's certificate filed by me on the same subject matter having a filing date before that of the application(s) from which priority is claimed.

Check one:

_X_ no such applications have been filed.

_ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

60/113,487	December 22, 1998
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)	(Filing Date)	(Status) (patented,pending,aband.)
(Application Serial No.)	(Filing Date)	(Status) (patented,pending,aband.)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

W. Hugo Liepmann	Reg. No. 20,407	Catherine J. Kara	Reg. No. 41,106
James E. Cockfield	Reg. No. 19,162	Jeanne M. DiGiorgio	Reg. No. 41,710
Thomas V. Smurzynski	Reg. No. 24,798	Megan E. Williams	Reg. No. 43,270
Ralph A. Loren	Reg. No. 29,325	Nicholas P. Triano III	Reg. No. 36,397
Giulio A. DeConti, Jr.	Reg. No. 31,503	Peter C. Lauro	Reg. No. 32,360
Ann Lamport Hammitte	Reg. No. 34,858	Timothy J. Douros	Reg. No. 41,716
Elizabeth A. Hanley	Reg. No. 33,505	John L. Welch	Reg. No. 28,129
Amy E. Mandragouras	Reg. No. 36,207	DeAnn F. Smith	Reg. No. 36,683
Anthony A. Laurentano	Reg. No. 38,220	William D. DeVaul	Reg. No. 42,483
Jane E. Remillard	Reg. No. 38,872	David J. Rikkers	Reg. No. 43,882
Jeremiah Lynch	Reg. No. 17,425	Chi Suk Kim	Reg. No. 42,728
Kevin J. Canning	Reg. No. 35,470		
David A. Lane, Jr.	Reg. No. 39,261		

Send Correspondence to Amy E. Mandragouras at **Customer Number: 000959** whose address is:

Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109

Direct Telephone Calls to: Amy E. Mandragouras , (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Winfried Edelmann
Inventor's signature Date
Residence 5800 Arlington Ave., Apt. 10H Bronx, New York 10471
Citizenship German
Post Office Address (if different)

Full name of second inventor, if any Richard D. Kolodner	
Inventor's signature Richard D. Kolodner	Date April 5, 2000
Residence 13468 Kibbings Rd. San Diego, California 92130	
Citizenship U.S.	
Post Office Address (if different)	

Full name of third inventor, if any Jeffrey W. Pollard	
Inventor's signature	Date
Residence 275 W. Ninety-sixth Street. New York, New York 10025	
Citizenship U.S.	
Post Office Address (if different)	

Full name of fourth inventor, if any Raju S. Kucherlapati	
Inventor's signature	Date
Residence Five Gracie Lane Darrien, Connecticut 06820	
Citizenship U.S.	
Post Office Address (if different)	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Winfried Edelmann, Richard D. Kolodner, Jeffrey W. Pollard, and Raju S. Kucherlapati

Art Unit:

Examiner:

Divisional of Serial No.: 09/469,636

Filed: Herewith

For: *METHODS FOR IDENTIFYING COMPOUNDS WHICH MODULATE THE ACTIVITY OF MSH5* (*As amended*)

Attorney Docket No.: AHN-001DV1

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: September 11, 2000 Mailing Label Number: EL 011 359 939 US

I hereby certify that this 37 CFR 1.53(b) request and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Viviato G. Carcoso — Name of Person Mailing Paper

Signature of Person Mailing Paper

ASSOCIATE POWER OF ATTORNEY

Sir:

The undersigned attorney has the power of attorney in the subject application. She hereby grants an associate power to:

Maria C. Laccotripe
LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109

As demonstrated by Appendix A (submitted herewith) Maria C. Laccotripe has passed the Patent Bar Examination and has been granted limited recognition under 37 C.F.R. § 10.9(b).

Please continue to forward all written and telephonic communications to Amy E. Mandragouras at the address and telephone number listed below.

Respectfully submitted,

Ann Lamport Hammitte
Registration No. 34,858
Attorney for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
Tel. (617) 227-7400
Dated: September 11, 2000

BEFORE THE OFFICE OF ENROLLMENT AND DISCIPLINE
UNITED STATE PATENT AND TRADEMARK OFFICE

LIMITED RECOGNITION UNDER 37 CFR § 10.9(b)

Maria C. Laccotripe is hereby given limited recognition under 37 CFR § 10.9(b) as an employee of Lahive and Cockfield to prepare and prosecute patent applications wherein the patent applicant is the client of Lahive and Cockfield, and the attorney or agent of record in the applications is a registered practitioner who is a member of Lahive and Cockfield. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to the date appearing below: (i) Maria C. Laccotripe ceases to lawfully reside in the United States, (ii) Maria C. Laccotripe's employment with Lahive and Cockfield ceases or is terminated, or (iii) Maria C. Laccotripe ceases to remain or reside in the United States on an H-1 visa.

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the U.S. Patent and Trademark Office.

Expires: October 5, 2000

Harry I. Moatz, Acting Director
Office of Enrollment and Discipline